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Expression of the MN Antigen in Cervical Papanicolaou Smears Is an Early Diagnostic Biomarker of Cervical Dysplasia¹

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Abstract

A new tumor-associated antigen, MN, has been shown to be expressed in virtually all cervical carcinomas and the majority of cervical intraepithelial neoplasia, but not in normal cervixes (S. Y. Liao *et al.*, *Am. J. Pathol.*, 145: 598-609, 1994). Therefore, we postulated that the exfoliative cells in cervical Papanicolaou (Pap) smears would reflect the MN immunoreactivity seen in the tissue sections, and high levels of MN expression in the exfoliative cells would indicate the presence of dysplasia in the cervix.

A total of 305 cervical Pap smears, with histological confirmation, representing all categories of the Bethesda System, were immunohistologically examined. We found that high levels of MN expression in exfoliative cells were not restricted to the dysplastic cells but were observed also in the normal endocervical cells (NECs) when dysplasia was present in the tissue biopsies. Overall, the rates of positive MN immunostaining of the dysplastic cells in low- and high-grade squamous intraepithelial lesions and invasive carcinoma were 35 (65%) of 54, 44 (77%) of 57, and 12 (92%) of 13, respectively. However, diffuse MN immunoreactivity of the atypical and/or dysplastic endocervical columnar cells was seen in all cases (100%) of adenocarcinoma *in situ* (AIS; *n* = 23) and adenocarcinomas (*n* = 8). In the groups with cytological diagnoses of atypical squamous cells or atypical glandular cells of undetermined significance (ASCUS and AGUS, respectively), MN positivity was seen in 47% of ASCUS (22/47) and 55% of AGUS (12/22). Dysplastic tissues were identified in all MN-positive cases. In contrast, all MN-negative atypical Pap smears were confirmed histologically to be benign cervix with one exception, in which the cytological diagnosis was ASCUS and focal low-grade squamous intraepithelial lesions were found in the cervix. The study also included 89 cases with cytological diagnoses of within normal limits/benign cellular changes. Among these, 10 Pap smears expressed

diffuse MN antigen in the NEC, and dysplasia (8 cases of low-grade squamous intraepithelial lesions, 2 AIS) was found in the cervixes. None of MN-negative cases with "within normal limits" cytology contained dysplastic cervixes. Therefore, it would seem that diffuse MN antigen expression in the NEC may be an indicator of cervical dysplasia. Thus, MN antigen might serve as an early biomarker of cervical neoplasia. The combination of detection via cytology and MN immunostaining resulted in no false negatives and also discriminated between cellular atypia due to benign reactive changes *versus* cellular atypia due to dysplasia in the category of ASCUS and AGUS. In particular, it was found in the AGUS group that diffuse MN immunostaining restricted to atypical columnar cells was diagnostic for AIS. These findings indicate that MN antigen expression is an important diagnostic biomarker of glandular neoplasia and a valuable adjunct to cytological diagnosis of ASCUS and AGUS.

Introduction

The final step of cancer prevention is to eradicate, if possible, the incidence of cancer mortality from invasive disease. It is now well accepted that neoplastic evolution embodies a multistage process that reflects multiple genetic events involving activation of oncogenes and loss of function of tumor suppressor genes (1-3). Thus, the search for surrogate end point biomarkers has become an important task, so that cancer cells can be detected before invasion occurs, or even identified as early as during the period of genomic instability prior to the microscopic appearance of neoplastic changes. Many such biomarkers have been described that reflect structural abnormalities of DNA, expression of tumor-associated protein and carbohydrate antigens, and detection of activated oncogene products or tumor suppressor gene defects. Despite the plethora of biomarkers reported, very few have proven to be useful for early detection of cervical cancer.

In the case of cervical cancer, the most effective diagnostic procedure is still based on the cytological examination of cervical Pap³ smears. Indeed, the wide acceptance of the Pap smear cytological screening program has resulted in dramatic decreases in the incidence of, and mortality from, cervical cancer in the United States over the past 40 years. However, significant rates of morbidity and mortality still exist (4). The cytology laboratory has been implicated frequently as the weak link in cervical cancer prevention efforts because of an inher-

Received 11/3/95; revised 3/15/96; accepted 4/4/96.

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¹ These studies were supported by NIH Grant CA19401.

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³ The abbreviations used are: Pap, Papanicolaou; NEC, normal endocervical cell; AIS, adenocarcinoma(s) *in situ*; ASCUS, atypical squamous cells of undetermined significance; AGUS, atypical glandular cells of undetermined significance; WNL, within normal limits; SIL, squamous intraepithelial lesion; LSIL, low-grade SIL; HSIL, high-grade SIL; CA, carcinoma; MAbs, monoclonal antibody; CIN, cervical intraepithelial neoplasia.

ently high false negative rate and inconsistent cytological reporting systems (5-7).

The Bethesda System conferences and the Clinical Laboratory Improvement Amendment of 1988, as well as the revision of 1991, have standardized the reporting system and improved communication between clinician and cytopathologist (8, 9). However, at the same time, the use of the term "atypical squamous cells or atypical glandular cells of undetermined significance" creates the greatest problem for the clinician who then must select the appropriate follow-up procedure and represents the larger volume of patients who require evaluation. Questions have also arisen repeatedly concerning the reliability of cytology or histology as a predictor of lesion behavior (10, 11).

A novel membrane antigen (MN) was described recently, and its expression has been associated with the tumorigenic phenotype of cervical adenocarcinoma HeLa cells and HeLa X fibroblast hybrids (12). The gene encoding the MN product is novel, the only identified functional domain to date being a carbonic anhydrase domain (13). A preliminary screen of clinical specimens indicated that the expression of the MN antigen is restricted to very few normal tissues, but significant levels of expression were noted in certain malignancies, including cervical cancer (12). In a recent study of several hundred benign and neoplastic cervical epithelial specimens, we have observed significant levels of MN expression in more than 90% of cervical neoplasms. A striking observation in this study was that morphologically normal reserve and/or columnar cells, normally MN antigen negative, showed strongly positive immunohistochemical staining in the regions adjacent to dysplastic lesions in approximately 40% of cases (14). Based on our studies of cervical biopsies, it would be our expectation that significant levels of MN expression seen in normal exfoliative cells would indicate the presence of dysplasia in the cervix. To explore this hypothesis, a total of 305 smears, representing the complete spectrum of the Bethesda System, were studied. Corresponding histological examination of biopsy material was conducted for each specimen. We find that MN antigen expression in exfoliative cells recapitulates MN expression in the tissue sections. Diffuse strong MN immunoreactivity of cells in the cervical smears appears to be associated always with dysplasia, regardless of whether the positive cells have the cytological appearance of NECs or dysplastic cells. Virtually all of the atypical and dysplastic columnar cells in the cytological smears derived from glandular dysplasia and *in situ* or invasive adenocarcinoma expressed high levels of MN antigen. These findings suggest strongly that MN antigen is indeed an early biomarker of dysplasia and has the potential to serve as a useful adjunct to cytological diagnosis.

Materials and Methods

Tissue Specimens. Three hundred five Pap smears from 305 patients were obtained from several medical centers. The median age of the patients was 42 years, and the ages ranged from 19 to 81 years. All of the cases except two were selected from private community and health maintenance organization hospitals in Southern California. The smears included in the study represented the full range of cytological categories of the Bethesda System, including: WNL, benign cellular changes, ASCUS, AGUS, LSIL, HSIL, AIS, and invasive CA. To simplify the terminology used in the study, benign cellular changes were included in the category of WNL. The WNL specimens were obtained from simple hysterectomies that were performed for reasons that included fibroidosis, endometriosis, chronic pelvic

pain, and pelvic discomfort. The WNL cases studied also included pregnant women, patients with trichomonas or herpes infections, and those receiving hormonal administration. The majority of smears were collected by both cervical scraping and cytobrush. Only those smears containing at least two clusters of well-preserved endocervical and/or metaplastic squamous cells, with each cluster composed of at least five appropriate individual cells, were included in the study. All of the cases included in the study also had histological confirmation concurrently or subsequently after the Pap smears were taken. The sources of tissue sections of the cervixes were directly from colposcopic-directed cervical biopsies, endocervical curettage, or cervical conization in the majority of the studied cases ($n = 203$). The CA specimens were obtained from radical hysterectomies ($n = 13$), and the remaining 89 cases (WNL) were obtained from simple hysterectomies.

Immunohistochemical Studies. A hybridoma secreting an anti-MN MAB, designated M75, was prepared from splenocytes of a mouse immunized with HeLa cells expressing the MN protein on their surface (12). Immunohistochemical staining of tissue sections and decolorized Pap smears with the anti-MN MAB was done using a peroxidase technique described previously (15). Known positive and negative tissue specimens were included in each staining run. Briefly, the smears were decolorized with 1% acid alcohol and rinsed with distilled water. Five- μ m sections of paraffin-embedded tissues were deparaffinized. The endogenous peroxidase was blocked by incubating the slides in a solution of 2.5% hydrogen peroxide in methanol for 45 min. The slides were then incubated with appropriate blocking serum (5% normal horse serum in PBS-containing 0.1% BSA) for 20 min. All incubations were performed at room temperature in humidified chambers. The slides were then incubated with purified ascites fluid-derived primary antibody M75 (1:550 dilution in PBS) for 60 min and then with secondary biotinylated horse antimouse immunoglobulin G antibody (1:200 dilution in PBS) for 60 min, followed by incubation with avidin-biotin peroxidase complexes (ABC Elite) for 30 min (Vector Laboratories, Burlingame, CA). Diaminobenzidine tetrahydrochloride was used as chromagen (Sigma Chemical Co., St. Louis, MO). After treatment, the sections were washed with distilled H₂O, counterstained with hematoxylin, and mounted with permount.

Specificity of staining was identified by the presence of a brown reaction product predominantly on the plasma membrane. The slides were initially scanned at the low-power magnification of 4 \times and scored as MN negative when no brown staining was seen and positive when dark brown product was easily detected. Weak staining, detectable only at a high-power magnification, was noted occasionally. The weak staining was primarily present in the cytoplasm and was noted occasionally in normal and dysplastic cells. In this study, it was included in the category of negative staining because of the lack of specificity. The distribution of MN immunostaining was semiquantitatively scored as diffuse when the majority of the endocervical and/or dysplastic cells (if present) were positive; focal when only isolated endocervical cell clusters and/or dysplastic cells (if present) were positive; and negative when no cell showed positive staining, or when positive immunostaining was restricted to only a few individual cells.

Results

Rescreening of Cervical Pap Smears. All of the cytological smears and tissue specimens examined in this study were obtained from archival sources that had already received a cyto-

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Table 1 Correlation between the original and rescreened cytological diagnoses of the cervical pap smears

Original diagnosis	Rescreened diagnosis						
	WNL	ASCUS	AGUS	LSIL	HSIL	AIS	CA
WNL (n = 102)	89	3	0	0	2	8	0
ASCUS (n = 51)	0	44	0	7	0	0	0
AGUS (n = 30)	0	0	22	0	0	8	0
LSIL (n = 47)	0	0	0	43	4	0	0
HSIL (n = 55)	0	0	0	4	51	0	0
AIS (n = 7)	0	0	0	0	0	7	0
CA (n = 13)	0	0	0	0	0	0	13

logical and histological diagnosis. For inclusion in the current study, all of the smears were rescreened. The original cytological diagnoses identified 102 smears classified as WNL and 51 ASCUS, 30 AGUS, 47 LSILs, 55 HSILs, 7 AIS, and 13 CAs. The rescreened cytological diagnoses identified 89 WNL smears and 47 ASCUS, 22 AGUS, 54 LSILs, 57 HSILs, 23 AIS, and 13 CAs. The comparative analyses and the identified discrepancies are summarized in Table 1. The results of this rescreening illustrate the problems of underdiagnosis in cytological screening and, in particular, the underdiagnosis of AIS.

Immunohistochemical Analysis. The smears representing all categories of the Bethesda System were immunostained with MAb to MN protein. Examples of negative specimens and the range of positive MN immunostaining are illustrated in Fig. 1. The exfoliative cells that expressed MN antigen were not restricted to dysplastic cells (Fig. 1, E-H) but were also observed in morphologically normal-looking endocervical components, such as reserve and columnar cells (Fig. 1, C and D). However, the normal superficial, intermediate, and parabasal cells were consistently negative. The presence of MN-positive reserve or columnar cells in the cytological smears was a critical indicator of dysplastic lesions. Whenever the normal exfoliative cells in the smears were MN negative (Fig. 1, A and B), the cervixes were histologically normal or benign. In contrast, when diffuse MN immunoreactivity was present in the reserve and/or columnar cells (Fig. 1, C and D), irrespective of the presence or absence of dysplastic cells, the cervixes contained dysplastic lesions. A detailed analysis of the various categories follows.

MN Expression in Pap Smears with Cytological Diagnoses of CAs. Thirteen cases with cytological diagnoses of CA were studied. All of these cases had radical hysterectomy performed. The histological diagnoses included two large-cell keratinizing and two large-cell nonkeratinizing squamous cell CAs, one adenosquamous cell CA, and eight adenocarcinomas of the endocervical type. Heterogeneous MN expression was noted in smears from the single case of adenosquamous cell CA and three squamous cell CAs. One large-cell keratinizing case was MN negative. Diffuse MN immunoreactivity was seen in smears and histological sections from all eight cases of adenocarcinoma (Fig. 2, A and B).

MN Expression in Pap Smears with Cytological Diagnoses of SIL and AIS. In this study, the original cytological diagnoses included 102 SIL (47 low grade, 55 high grade) and 7 AIS. The rescreened cytological diagnoses identified 111 SILs (54 LSILs, 57 HSILs) and 23 AIS. The results of the immunostaining are summarized in Table 2. All 23 smears identified as AIS were subsequently confirmed histologically in tissue biopsies.

In the SIL cases, MN protein expression was seen in dysplastic and morphologically normal endocervical columnar and/or reserve cells in the Pap smears (Fig. 2C). The numbers

of cells stained varied in each case, ranging from a few cells being positive to almost all of the dysplastic and/or endocervical cells exhibiting immunoreactivity (Fig. 2, C-E). Of 54 Pap smears with rescreened cytological diagnoses of LSIL, 19 either were MN negative or showed focal positivity of the normal-looking endocervical cells. However, the dysplastic cells were MN negative. Coexpression of MN antigen in dysplastic/metaplastic cells, reserve, and/or columnar cells was seen in the remaining 35 smears. Diffuse MN expression was noted in 22 smears, primarily in the endocervical cells and to a lesser degree in the dysplastic cells. Of the 57 Pap smears with rescreened cytological diagnoses of HSIL, variable immunoreactivity was seen. Coexpression of MN antigen in both dysplastic cells and normal endocervical components was seen in 44 smears, including diffuse MN immunoreactivity of dysplastic cells in 21 smears. The remaining 13 smears either were MN negative or exhibited focal positivity restricted to isolated endocervical cell clusters.

Overall, the rates of positive immunostaining of dysplastic cells in LSILs and HSILs were 65 and 77%, respectively. Among these, 37% of HSILs and 41% of LSILs showed high levels of MN expression. In those cases where diffuse MN immunoreactivity was seen in the Pap smears, the immunostaining of the corresponding cervical biopsies also showed positive staining distributed in all layers of dysplastic cells or in the intermediate and superficial layers of the dysplastic tissues (Fig. 2, E and F). In those cases where the exfoliative dysplastic cells were MN negative or exhibited focal positivity, low levels of MN expression were seen in the tissue sections in which positive staining was limited to a few dysplastic cells or to the intermediate layer of the dysplastic tissues. Morphologically, most of these MN-negative dysplastic cells were either koilocytotic or differentiated keratinizing squamous cells (Fig. 2, G and H). With respect to the high levels of coexpressed MN antigen seen in the dysplastic and normal-looking endocervical cells, the relative distributions of MN-positive cells in LSILs and HSILs were significantly different. In LSILs, MN positivity was seen in the majority of morphologically normal columnar/reserve or metaplastic cells but was expressed only in limited numbers of dysplastic cells. In contrast, most of the MN-immunoreactive cells in HSILs were dysplastic cells. The percentage of the cases with diffuse MN immunostaining in the NECs was 9 versus 41% for HSILs and LSILs, respectively.

The Pap smears of all AIS cases in the study were MN positive. Virtually all of the dysplastic and/or atypical endocervical glandular cells seen in the smears expressed diffuse strong plasma membrane staining for MN antigen (Fig. 1, G and H). In contrast to SIL cases, the normal columnar and reserve cells were negative. There were two exceptions that showed diffuse MN staining of NECs. In these two cases, coexisting LSIL lesions were identified in the tissue sections.

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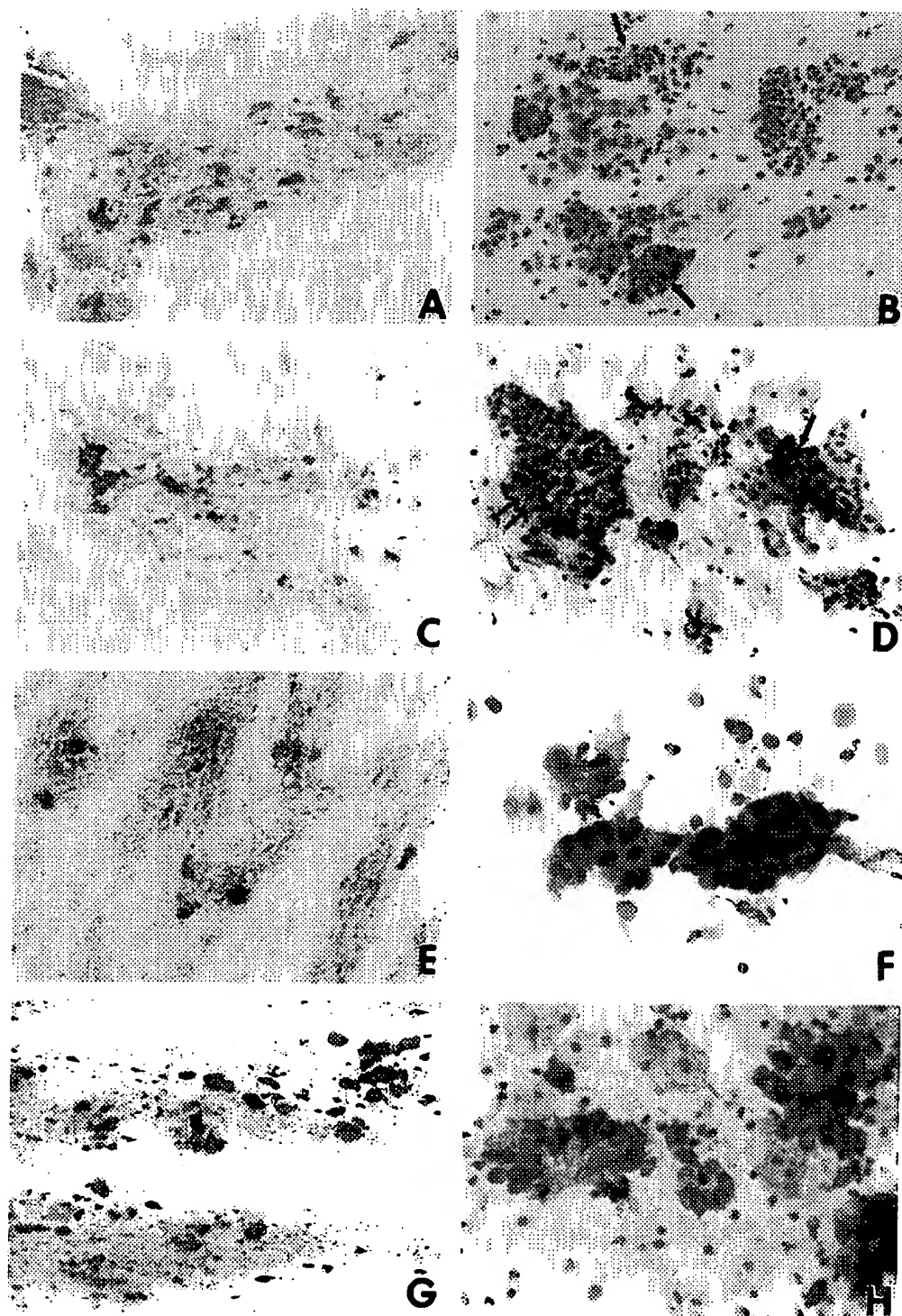


Fig. 1. MN Antigen immunoreactivity in cervical Pap smears. Normal Pap smears show no immunoreactivity (A) or nonspecific weak intracytoplasmic immunoreactivity restricted to a few reserve/columnar cells (arrows) at a high-power magnification (B). C, Pap smear from a diagnosed case of ASCUS. Diffuse MN immunoreactivity is seen in cell clusters at a low-power magnification. At the high-power magnification (D), it is seen that the strong immunoreactivity is located particularly at the plasma membranes of reserve cell (single arrow) and endocervical columnar cell clusters (double arrow). No dysplastic cell is seen in this smear. E and F, smears of HSIL case. Diffuse immunoreactivity is seen at low power (E), and the positively staining cells are seen to be dysplastic cells (F). In Pap smears of AIS, practically all of the dysplastic glandular cells and cell clusters exhibit diffuse immunoreactivity (G) with strong plasma membrane immunoreactivity seen at the higher magnification (H). The original magnifications are as follows: A, C, E, and G $\times 40$; B and D, $\times 200$; F and H, $\times 400$.

Fig. 2 Comparing a patient with cervical normal endocervical invasive areas (A) (C). The pattern of exfoliative dysplasia shows low levels of immunoreactivity as follows: A and

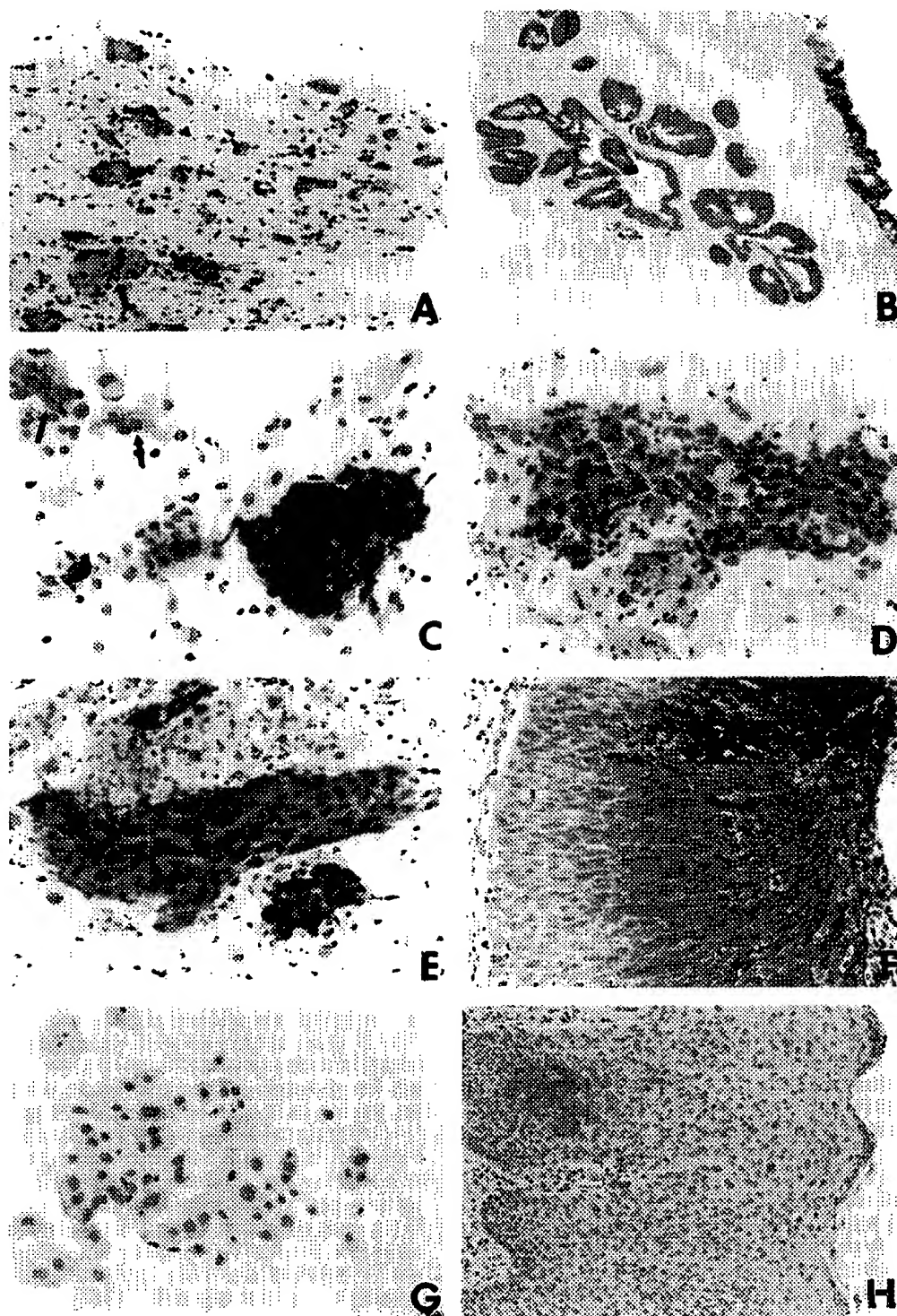


Fig. 2 Comparative MN antigen immunoreactivity between Pap smears and the corresponding tissue sections of cervical adenocarcinoma and CIN. In specimens from a patient with cervical adenocarcinoma, the Pap smear shows diffuse immunoreactivity of practically all of the exfoliative dysplastic and atypical columnar cells (A). The normal endocervical columnar cells are negative (arrow). The corresponding histology of the adenocarcinoma also shows diffuse immunoreactivity of both the *in situ* and invasive areas (B). In cases of CIN (SIL), immunoreactivity in Pap smears may involve both dysplastic cell clusters and normal-looking columnar cells, shown by the arrows (C). The pattern of immunoreactivity may be focal (D) or diffuse (E). The corresponding dysplastic tissue show diffuse immunoreactivity (F). In those smears where the exfoliative dysplastic cells are MN negative, they are often koilocytotic or a differentiated keratinizing type (G). In these cases, the corresponding dysplastic tissues show low levels of immunoreactivity limited to a few dysplastic cells and often localized to the intermediate layer of the dysplastic tissue (H). The original magnifications are as follows: A and B, $\times 100$; C, $\times 400$; D-H, $\times 200$.

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Table 2 Expression of the MN antigen in exfoliative dysplastic cells of squamous and glandular intraepithelial neoplasia

Cytological diagnosis	No. of smears examined	No. of positive smears
LSIL	54	35 (65) ^a
HSIL	57	44 (77)
AIS	23	23 (100)

^a Percentages are shown in parentheses.

MN Expression in Pap Smears with Cytological Diagnoses of WNL. A total of 102 Pap smears with original cytological diagnoses of WNL were included in this study, and the results are summarized in Table 3. Rescreening of the smears led to reclassification of 3 cases as ASCUS, 2 as HSILs, and 16 as AIS (Table 1). On the basis of the rescreened cytological diagnoses, 61 out of 89 Pap smears were MN negative, and 18 smears exhibited a focal pattern of MN immunoreactivity. Diffuse MN immunoreactivity was seen in the remaining 10 normal Pap smears as well as the 13 abnormal smears (3 ASCUS, 2 HSILs, and 8 AIS) that had been originally diagnosed as WNL. Dysplasia was identified in the tissue sections of all cases where the corresponding smears showed diffuse MN immunoreactivity, irrespective of cytological diagnosis. As noted previously, diffuse MN staining of NECs was always associated with SILs.

Thus, in each case, diffuse MN immunostaining of cells in the Pap smears was diagnostic of a dysplastic lesion. It should particularly be noted that diffuse MN immunoreactivity was seen in 23 of the 102 Pap smears diagnosed originally as WNL. Even after rescreening, 10 cases with a diagnosis of WNL were MN positive, and in all 10 cases, dysplastic lesions were confirmed by biopsy.

MN Expression in Pap Smears with Cytological Diagnoses of ASCUS and AGUS. Originally, there was a total of 51 Pap smears with cytological diagnoses of ASCUS. The rescreened cytological diagnoses identified 7 LSILs and 44 ASCUS. In addition, there were three smears with rescreened cytological diagnoses of ASCUS from the WNL category (Table 1). Of the total of 47 smears, 25 were MN negative, and variable degrees of MN immunoreactivity were seen in the remaining 22 smears (Table 4). All seven smears with rescreened cytological diagnoses of LSIL expressed the MN protein. MN-positive smears, regardless of whether the staining patterns were diffuse or focal, all correlated with a diagnosis of dysplasia in biopsies of cervical tissue. Dysplasia was found in only one case where the smear was scored as MN negative. In this one exception, the dysplasia was focal and low grade.

There was a total of 30 Pap smears with original diagnoses of AGUS included in the study. The rescreened cytological diagnoses identified eight AIS from this category (Table 1). Of the 22 smears with rescreened cytological diagnoses of AGUS, 10 were MN negative, and 6 showed focal positivity. Diffuse, intense MN immunoreactivity in morphologically atypical endocervical columnar cells was observed in the remaining six cases, as well as the eight cases of AIS that were originally diagnosed as AGUS. All of the MN-negative cases were confirmed histologically to be benign cervixes with reparative changes. In contrast, SILs and AIS were identified in all of the tissue biopsies that corresponded to those smears that expressed MN protein.

The Correlation between Histopathology and MN Immunoreactivity of Cervical Pap Smears. When the results of MN immunoreactivity and the cytological diagnoses were com-

Table 3 MN immunoreactivity in pap smears with cytological diagnoses of WNL

MN immunostaining	Rescreened cytological diagnoses			
	WNL <i>n</i> = 89	ASCUS <i>n</i> = 3	HSIL <i>n</i> = 2	AIS <i>n</i> = 8
Negative	61 (69) ^a	0	0	0
Positive with focal staining ^b	18 (20)	0	0	0
Positive with diffuse staining pattern ^c	10 (11)	3 (100)	2 (100)	8 (100)

^a Percentages are shown in parentheses.^b Cervical biopsies of all cases showed histological evidence of reserve cell hyperplasia with variable degrees of cytological atypia.^c Cervical biopsies of all cases showed histological evidence of dysplasia.

Table 4 MN immunoreactivity in pap smears with cytological diagnoses of ASCUS and AGUS

MN immunostaining	Rescreened cytological diagnoses			
	ASCUS <i>n</i> = 47	AGUS <i>n</i> = 22	LSIL <i>n</i> = 7	AIS <i>n</i> = 8
Negative	25 (53) ^a	10 (45)	0	0
Total number of positive smears ^b	22 (47)	12 (55)	7 (100)	8 (100)

^a Percentages are shown in parentheses.^b All MN-positive smears were histologically confirmed to be dysplasia (see Table 5).

pared with the histopathology of the cervix, it became apparent that diffuse MN immunoreactivity of morphologically normal exfoliative cells was indicative of dysplasia (Table 5). In the 10 cases with rescreened cytological diagnoses of WNL that exhibited high levels of MN expression, tissue sections from cervical biopsy or hysterectomy specimens contained LSILs (*n* = 8) and minimal AIS (*n* = 2). Reserve cell hyperplasia and/or immature squamous metaplasia, accompanied with variable degrees of cellular atypia, was noted in the 18 cases in which relatively few MN-positive cells were seen in the smears. In all cases (*n* = 61) where the staining pattern was negative, the cervixes either were normal or showed cervicitis with squamous metaplasia. In the group with rescreened cytological diagnoses of ASCUS or AGUS, dysplastic tissues were identified in all cases where MN-positive endocervical cells were seen in the Pap smears. In contrast, the cytologically diagnosed ASCUS or AGUS cases that showed no evidence of MN immunostaining correlated to benign histology of the cervixes, with one exception. In this latter case, focal condyloma was diagnosed in the cervical biopsy specimen. One of the significant observations of this study is that in all of the Pap smears, even with rescreened cytological diagnoses of WNL and AGUS, diffuse MN immunostaining of the exfoliative atypical columnar cells was always associated with AIS in the cervixes. In the case of SIL, the number of dysplastic cells stained appeared to be related closely to the status of cell differentiation and the extent of the cervix involved with dysplasia. The majority of dysplastic cells seen in MN-negative smears were koilocytotic cells or differentiated keratinizing squamous cells (Fig. 2G). In these cases, the dysplasia present in the cervix either was minimal in degree and extent or exhibited histological features of flat condyloma and/or differentiated dysplasia (Fig. 2H). In contrast, most of the dysplastic cells expressing the MN antigen were derived from an immature form of dysplasia (Fig. 2, E and F), and often they were cytologically indistinguishable from basaloid, atypical glandular, or imma-

Table 5

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Table 5 The correlation between rescreened cytological diagnoses, MN antigen expression, and the corresponding histological diagnosis of the cervix

Rescreened cytological diagnosis	Histological diagnosis and MN antigen expression No. of cases (No. MN positive)					
	Normal	RCH/ISM ^a	LSIL	HSIL	AIS	CA
WNL (n = 89)	61 (0)	18 (18) ^b	8 (8)	0	2 (2)	0
ASCUS (n = 47)	24 (0)	0	23 (22) ^c	0	0	0
AGUS (n = 22)	10 (0)	0	4 (4)	2 (2)	6 (6)	0
LSIL (n = 54)	0	0	54 (35)	0	0	0
HSIL (n = 57)	0	0	0	57 (44)	0	0
AIS (n = 23)	0	0	0	0	23 (23)	0
CA (n = 13)	0	0	0	0	0	13 (12)

^aRCH/ISM: Reserve cell hyperplasia/immature squamous metaplasia.

^bVariable degrees of cellular atypia were noted in the reserve or immature squamous cells.

^cOne MN-negative LSIL case in which the cervix shows minimal condyloma.

more metaplastic cells. In those smears that showed MN expression in morphologically normal endocervical columnar cells, there was a frequent correlation with a histology of glandular atypia associated with squamous dysplasia of the cervix. In contrast, when MN-immunoreactive columnar cells were cytologically abnormal, glandular dysplasia or AIS was found in the tissue sections.

Discussion

A stepwise interrelationship between precancerous lesions and invasive cervical CA has been postulated by several investigators (16–20). Theoretically, early detection of precancerous lesions by massive cancer screening programs based on the use of the cervical Pap smear could result in a total prevention of invasive malignant disease. Yet, despite the wide acceptance of the screening program in 1991, a survey from the United States (4) showed an estimated 13,000 women were diagnosed with invasive CA, and an estimated 4500 women died of the disease in that year. In addition, the relative frequency of adenocarcinoma of the cervix has tripled in the past two decades (21).

Thus, there are clearly deficiencies in the current system of screening. One of the major concerns is the inherently high rate of false negatives. Reports have indicated that the false negative rate has remained in the range of 5% upon rescreening of cervical smears (7, 22, 23). The results appear to indicate that the errors may be based primarily on human factors, which are not remedied readily by rules and regulations (23). Other concerns include the reliability of cytology and the binary (Bethesda) reporting system as a predictor of lesion behavior (10, 11). Thus, it appears that there is a need for supplementary cervical cancer screening techniques. We show here that detection of the tumor-associated MN antigen is such a useful adjunct.

In our earlier investigation of the immunohistochemical distribution of MN antigen in normal and neoplastic tissues of the cervix, we showed that MN antigen is expressed in 100% of AIS, and in more than 90% of CAs and CIN lesions but is not expressed in normal cervical tissues (14). However, concurrent with the presence of CIN, AIS, and CA, an increasing number of normal reserve and/or columnar cells were shown to express the MN antigen and to exhibit features of reserve cell hyperplasia (14). Therefore, we postulated that the exfoliative cells in Pap smears would reflect the MN immunoreactivity seen in the tissue sections. This study confirms that postulation. Regardless of cytological diagnosis, the normal superficial, intermediate, and parabasal cells in the smears are consistently MN negative. Variable degrees of MN expression are seen in the normal endocervical components, and this expression is related closely

to the status of reserve cell proliferation and the presence or absence of cellular atypia or dysplasia of both glandular and squamous cell origin. For example, when the cervix is histologically normal without associated reserve cell hyperproliferation, the exfoliative cells are MN negative. Only in those cases where there is concurrent reserve cell and/or atypical glandular hyperplasia is MN immunoreactivity seen in the smears. There is a strong association between the number of reserve and columnar cells that stained and the degree of cellular atypia. Furthermore, when diffuse MN immunoreactivity is seen, dysplastic lesions are found in the cervix.

Recent investigations have provided compelling evidence that subcolumnar reserve cells are facultative progenitor cells with the capacity to proliferate and differentiate into either columnar or metaplastic squamous cells (24, 25). We postulate that the variable degree of MN expression seen in the exfoliative reserve and columnar cells may reflect a very early stage of preneoplastic cellular alteration before dysplastic morphology is microscopically identified in the tissue. This hypothesis is supported by our observation of heterogeneity of MN expression in those cases where the cervixes show reserve cell hyperproliferation. In this study, we have found when the MN immunoreactivity was restricted to a few reserve cells, no appreciable cellular alteration was noted. However, when the MN-positive staining was distributed diffusely to the majority of endocervical cells, virtually all of the cervical tissues showed evidence of dysplasia.

The linkage of diffuse MN staining pattern in normal-looking endocervical cells to SIL is an intriguing observation, and its clinical implication may be significant. Recent studies have indicated that the presence of abnormal metaplastic cells or endocervical cells sometimes is the only indicator of associated SILs. However, these atypical cells are not separated readily from the benign reactive or reparative cells (26, 27). In this study, we found that 47% of the smears with rescreened cytological diagnoses of ASCUS, and 27% of smears diagnosed as AGUS were subsequently confirmed histologically as SIL. The majority of those smears exhibited diffuse MN expression in morphologically normal or abnormal endocervical components. In contrast, the cervical biopsies of all of the MN-negative Pap smears with cytological diagnoses of ASCUS or AGUS showed cervicitis with or without reparative changes, with one exception. Thus, it appears that the MN antigen may serve as an important biomarker for discrimination of those atypical cells associated with dysplasia *versus* atypical cells due to reactive or reparative changes of the cervix.

It should be noted that the rate of MN-positive staining of

SILs in the Pap smears is significantly lower than the rate of positivity seen in the tissue sections. These discrepancies can possibly be attributed to the fact that, in a significant proportion of the cases in which the MN-negative smears contained a few dysplastic cells, minimal disease involving the cervix was also seen. The histology of these MN-negative cases is predominantly that of flat condyloma and/or differentiated keratinizing dysplasia. Nevertheless, most of the MN-negative dysplastic cells exhibit characteristic cytological features of dysplasia and thus pose no cytological diagnostic difficulty. As indicated in this study, all of the cases with MN-negative dysplastic cells were diagnosed cytologically as SIL except one case of ASCUS. In contrast, all of the MN-negative, cytologically normal smears have histologically normal cervixes. Thus, with the combination of MN immunoreactivity and cytological diagnosis, there is no false negative diagnosis in this study.

Because of the increasing incidence of adenocarcinoma, coupled with the use of improved endocervical brush technology, it has become essential to clearly differentiate dysplastic glandular cells from atypical cells due to a reactive-reparative process or to low-grade glandular atypia associated with SIL. To date, no firm criteria have been established for the colposcopic findings of endocervical glandular dysplasia, AIS, and early invasive adenocarcinoma. Hence, both the histopathologist and cytopathologist bear the responsibility for diagnosis. Morphological criteria relating to benign or malignant endocervical glandular lesions have been described in recent years (28-30). However, controversy regarding endocervical glandular abnormalities persists. This uncertainty has increased confusion for the clinicians, who then have to select the appropriate follow-up procedure. This confusion has been recognized by Pacey *et al.* (30), who state, "until our collective experience in the cytological diagnosis of adenocarcinoma (of the cervix) and its precursors is greater, errors of prediction are inevitable." Indeed, in a number of studies reported in the literature, it was found that up to 48% of patients did not receive a diagnosis of AIS on the basis of Pap smears and/or endocervical curettage prior to conization of the cervix (31-34). In our own study, reported here, we also note a similarly high rate of cytological underdiagnosis. Only 15 of 23 patients with histologically confirmed AIS had Pap smears that were cytologically diagnosed as AIS ($n = 7$) or AGUS with suspicion of glandular dysplasia ($n = 8$) prior to tissue biopsy (Table 1). The reason, in part, was that the "underdiagnoses" were due to a combination of inflammation and/or poor fixation that obscured the dysplastic cells, a problem probably associated in general with misdiagnosis of AIS. We found that, regardless of cytological diagnosis or the quality of the smears, almost all of the dysplastic and atypical glandular cells in all cases of invasive adenocarcinoma and AIS exhibited diffuse MN immunostaining. Although additional study is necessary to determine the relationship between glandular dysplasia/atypia, AIS, and adenocarcinoma, the current data clearly indicate that MN antigen expression is a powerful diagnostic biomarker for glandular dysplasia and neoplasia.

Furthermore, although the number of invasive CAs examined was too small to be of significance, diffuse MN immunoreactivity was noted in all of the invasive adenocarcinomas screened.

We conclude the following. (a) MN expression seen in exfoliative cells in the Pap smear recapitulates MN expression in the corresponding tissue sections of the cervix. (b) MN immunostaining of a minority of normal cells is indicative of reserve cell hyperplasia with/without atypia. (c) Regardless of the cytological diagnosis, MN immunoreactivity in the majority of normal endocervical components appears to be indicative of

the presence of squamous dysplasia. (d) MN expression is related closely to the immature form of dysplasia, and high levels of MN expression in LSIL may be a prognostic indicator of the probability that the dysplastic cells may progress to a higher-grade lesion. (e) Virtually all dysplastic glandular cells in the Pap smears express the MN antigen. Thus, MN antigen expression would appear to be an important diagnostic biomarker for glandular dysplasia, AIS, and invasive adenocarcinoma. (f) Our data indicate that MN antigen expression in "normal" cells appears to be a useful early biomarker of dysplasia, and as such, might serve as a valuable adjunct to cytological diagnosis, particularly in the "gray" areas of ASCUS and AGUS.

Acknowledgments

We thank Drs. Scott McMeekin, Philip DiSaia, and Yao-S. Fu for helpful discussions. We are grateful to Dr. Jan Závada for the M75 MAB.

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